# **Endogenous Factors Affecting Oxidative** Stability of Beef Loin, Pork Loin, and Chicken Breast and Thigh Meats

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ABSTRACT: The susceptibility of meats from different animal species (chicken breast [CB] and thigh [CT], pork [PL], and beef [BL]) to lipid oxidation was studied. The amounts of TBARS in raw PL, CB, and CT did not change during a 7-d storage period. TBARS values of raw BL, however, significantly increased during 7-d storage because of high heme iron content, high lipoxygenase-like activities, and low 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities. Ferric ion reducing capacities (FRC) were detected in all raw meats, but their characteristics were different: storage-unstable in CB and CT and storage-stable in PL and BL. Ferric ion reducing capacities in raw CB and CT was higher than those of PL and BL, and could be related to their high oxidative stability. The TBARS values of cooked meat increased significantly with storage. The rates of TBARS increase in cooked CT and BL were significantly higher than those of cooked CB and PL after a 7-d storage. Nonheme iron content in cooked BL was higher than other meats and increased significantly after 7 d. Cooked BL had a higher amount of heat-stable FRC, which acted as a prooxidant in the presence of high free ionic irons, than other meats. Therefore, high heat-stable FRC and increased nonheme iron content in cooked BL were responsible for its high susceptibility to lipid oxidation. Despite relatively low nonheme iron and heat-stable FRC levels, cooked CT showed similar levels of TBARS to cooked BL after a 7-d storage because of its high PUFA content.

Keywords: DPPH radical scavenging activity, ferric ion reducing capacity, iron, lipid oxidation, lipoxygenase-like

# Introduction

ipid oxidation is one of the primary causes of quality deteriora-Ition in meat and meat products, and it generates compounds possibly detrimental to human health (Addis 1986; Kanner 1994). The conversion of muscle to edible meat after slaughter destroys the balance between prooxidative and antioxidative factors, resulting in initiation and propagation of lipid oxidation (Kanner 1994; Decker and Xu 1998; Morrissey and others 1998; Min and Ahn 2005).

The susceptibility of raw meat to lipid oxidation varies depending on animal species and muscle type (Rhee and Ziprin 1987; Salih and others 1989; Rhee and others 1996). Among meats, beef is the most susceptible to lipid oxidation (Kim and others 2002). Rhee and Ziprin (1987) and Rhee and others (1996) suggested that differences in heme pigment content and catalase activity determined the rate of lipid oxidation in raw meat. They hypothesized that meats with higher heme pigment content (beef) produce more hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) during oxymyoglobin autoxidation than meats with less heme pigments. Hydrogen peroxide can react with metmyoglobin to generate ferrylmyoglobin, which can initiate lipid oxidation (Kanner and Harel 1985; Egawa and others 2000; Baron and Andersen 2002). In addition to various iron catalysts, differences in fat content, fatty acid composition (Allen and Foegeding 1981), endogenous antioxidants such as carnosine and related dipeptides

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(Chan and Decker 1994), and antioxidant enzymes (Mei and others 1994) may also play important roles in oxidative stability of meat. Reducing compounds such as ascorbic acid can serve as electron donors in free radical-mediated oxidative processes (Buettner and Jurkiewicz 1996) and play a critical role in the progress of lipid oxidation. Ascorbic acid can serve either as an antioxidant or a prooxidant, depending on relative concentrations of ascorbic acid and the amount of iron present (Decker and Hultin 1992). Lipoxygenase is the major enzyme initiator of lipid oxidation in fish tissues (Saeed and Howell 2001), but it is also found in various mammalian tissues (Grossman and others 1988; Yamamoto 1992; Gata and others 1996). Lipoxygenase is capable of direct oxygenation of PUFAs, even in phospholipids (PLs) bound to membranes to generate lipid hydroperoxides.

Heating accelerates lipid oxidation and production of volatiles in meat (Han and others 1995; Byrne and others 2002; Beltran and others 2003) by disrupting muscle cell structure, inactivating antioxidant enzymes and other antioxidant compounds, and releasing iron from heme pigments (Kanner 1994; Mei and others 1994). High temperature causes reduction of activation energy for lipid oxidation and decomposes preformed hydroperoxides to free radicals, which stimulates autoxidation processes and off-flavor development (Min and Ahn 2005).

Although many suggestions have been made to explain the differences in oxidative susceptibility among meats from several animal species, no attempt has been made to compare various prooxidant and antioxidant factors among meats from different animal species. The objective of this study was to determine endogenous factors influencing oxidative stability of meats from 3 different animal species. The effect of heat treatments on the pro- and antioxidant factors in meats was also examined.

# **Materials and Methods**

# Sample preparation

Beef and pork loin muscles from 4 different animals were purchased from a local packing plant. Loins from each animal were used as a replication. Four replications were prepared. Chicken breast and thigh muscles were obtained from broilers raised in the Poultry Farm at Iowa State Univ. A total of 16 broilers (6 wk old) were slaughtered. Breast and thigh muscles from 4 birds were pooled and used as a replication. Muscles for each replication were ground separately, and patties (60 g) were prepared after grinding them twice through an 8-mm plate. The patties were individually packaged in oxygen-permeable zipper bags (polyethylene, 4 × 6, 2 mil; Associate Bag Co., Milwaukee, Wis., U.S.A.). Onehalf of the packaged patties were cooked in a bag in a 95 °C water bath to an internal temperature of 75 °C followed by cooling for 2 h at 4 °C. After draining meat juices from the bag, the patties were repackaged in oxygen-permeable bags. Raw and cooked patties were stored at 4 °C until used. Lipid oxidation, nonheme iron, ferric ion reducing capacity, free radical scavenging activity, and lipoxygenase activity of meat samples were determined at 0, 3, and 7 d of storage. Total iron content and fatty acid compositions of total fat, triglyceride (TG), phosphatidylcholine (PC), and phosphatidylethanolamine (PE) were determined at day 0.

# Chemical analyses

Lipid oxidation was determined by the method of Ahn and others (1998), and the amounts of 2-thiobarbituric acid-reactive substances (TBARS) were expressed as milligrams of malondialdehyde (MDA) per kilogram meat. Total fat content and fatty acid composition of total fat and lipid classes (triglyceride, phosphatidylcholine, and phosphatidylethanolamine) were determined by the method of Ahn and others (1995). Nonheme iron content was determined by the ferrozine iron analysis method (Carter 1971; Ahn and others 1993b). Total iron content was measured by the wet-ashing method of Carpenter and Clark (1995) and the ferrozine method of Ahn and others (1993b) with minor modifications. Meat sample (about 2 g) was accurately weighed into a 125-mL Erlenmeyer flask and mixed with 20 mL concentrated nitric acid. The mixture was placed at room temperature overnight for predigestion. The predigested solution was heated on a hot plate (150  $^{\circ}$ C) until dry. Three milliliters of Caro's acid (50% hydrogen peroxide:concentrated sulfuric acid = 4:1; Hatch and others 1985) containing peroxymonosulfuric acid were added to the flask and left on the hot plate until all peroxide was evaporated. After being cooled, the digest was transferred to a 10-mL volumetric flask using 0.01 N HCl as a rinse. The digest solution (0.5 mL) and 0.01 N HCl (1 mL) were added to a disposable test tube, mixed with 0.5 mL of 1% ascorbic acid in 0.2 N HCl (w/v), and left at room temperature for 5 min. To the test tube, 0.8 mL of 30% ammonium acetate (w/v) and 0.2 mL of ferrozine color reagent were added and thoroughly mixed. After 10 min, the absorbance of the mixture was determined at 562 nm against a blank. Heme iron content was estimated by the difference between total and nonheme iron contents.

Ferric ion reducing capacity (FRC) was determined using the method of Kanner and others (1991). Briefly, the meat homogenate (1 mL) prepared with 4 volumes of deionized distilled water (DDW) was mixed with 1 mM ferric chloride solution (1 mL) and stirred for 2 min at room temperature. After adding 11.3% trichloroacetic acid (TCA) solution (1 mL), the mixture was centrifuged at  $10000 \times g$  for 10 min. The supernatant (2 mL) was reacted with 0.8 mL of 10% ammonium acetate and 0.2 mL ferrozine color reagent for 10 min. The absorbance of the mixture was read at 562 nm against a blank.

Ferric ion reducing capacity (FRC) was expressed as micrograms of ascorbic acid equivalent per gram of meat.

Free radical scavenging activities of meat were determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (Saiga and others 2003). Ground meat (5 g) was homogenized with 15 mL of 75% ethanol solution (v/v) using a Polytron (Type PT 10/35; Brinkman Instrument Inc., Westbury, N.Y., U.S.A.) for 10 s at top speed and centrifuged at  $3000 \times g$  for 15 min. After being filtered through Whatman nr 1 filter paper (Whatman Intl. Ltd., Maidstone, U.K.), the filtrate was mixed with 1 mM DPPH radical in 100% ethanol at a 4:1 ratio (v/v). The mixture was thoroughly mixed and placed at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm. Sesamol (Sigma-Aldrich, St. Louis, Mo., U.S.A.) was used as a reference antioxidant. The residual DPPH radical was calculated by the following equation:

scavenged radicals (%) =

 $\frac{\text{(DPPH blank} + \text{control sample}) - \text{DPPH sample}}{\text{DPPH blank}} \times 100$ 

where DPPH blank is the absorbance of 75% ethanol solution (4 mL)/mL of DPPH solution, DPPH sample is the absorbance of sample solution (4 mL)/mL of DPPH solution, and control sample is the absorbance of sample solution (4 mL)/mL of 100% ethanol.

Lipoxygenase activity of meat was measured by a modified method of Gata and others (1996). The substrate solution was prepared by mixing 70 mg pure linoleic acid, 70 mg Tween-20, and 4 mL DDW. The solution was clarified by adding 0.5 mL of 1 N NaOH, and then diluted with DDW to 25 mL. The substrate solution was flushed with nitrogen gas and kept under nitrogen. Ground meat (5 g) was homogenized with 15 mL of 50 mM acetate buffer (pH 5.8) using a Polytron for 10 s at top speed, and then centrifuged at  $12000 \times g$  at 4 °C for 15 min. The supernatant was filtered through a Whatman nr 1 filter paper and the filtrate was used to determine lipoxygenase activities in meat. Lipoxygenase activity was assessed at 27 °C by the increase of absorbance at 234 nm produced by the formation of conjugated diene from linoleic acid. The reaction mixture was composed of 80  $\mu$ L sodium linoleate solution (10 mM), 80  $\mu$ L enzyme solution, and 50 mM acetate buffer (pH 5.8) to a final volume of 1 mL. The results were expressed as units of activity (U) per gram of meat, and calculated from the molar extinction coefficient of hydroperoxyl linoleic acid ( $\varepsilon = 25000 \, \mathrm{M}^{-1} \mathrm{cm}^{-1}$ ). One unit of lipoxygenase activity was defined as the amount of enzyme catalyzing the formation of 1  $\mu$ mol of hydroperoxide per minute.

# Statistical analysis

Data were analyzed using SAS (SAS Inst. 1989) and reported as means and standard error of means (SEM). Tukey's method (P < 0.05) was used to compare the mean values among treatments (Kuehl 2000).

# **Results and Discussion**

# Lipid oxidation

The initial (day 0) TBARS values for all raw meats from different animal species were not different from each other. At days 3 and 7, however, the TBARS values of beef loin were significantly higher than those of pork loin, chicken breast, and chicken thigh meats, and the rate of TBARS increase among raw meats during storage was highest in beef loin. The TBARS values of raw pork loin, chicken breast, and chicken thigh meat did not increase significantly during the 7-d storage (Figure 1A). The pattern of TBARS changes during storage appeared to be consistent with other studies (Rhee and

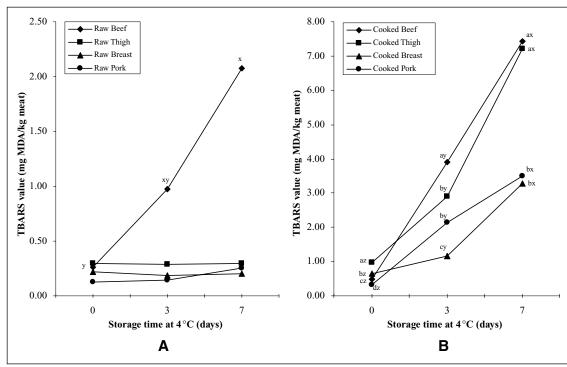


Figure 1 - TBARS values of raw (A) and cooked (B) chicken breast, chicken thigh, pork loin, and beef loin during storage at 4 °C (mg MDA/kg meat). Means with different letters (a to d) within meats from different animal species are significantly different (P < 0.05)**Means with** different letters (x to z) within a storage period are significantly different (P < 0.05). n = 4.

Ziprin 1987; Rhee and others 1996; Kim and others 2002), which reported that beef was the most susceptible to lipid oxidation among meats from different animal species. Rhee and others (1996) suggested that the difference in heme pigment content, which is associated with catalase activity, could be responsible for the difference in oxidative stability among meats from different animal species.

In cooked meat, the TBARS values of all meats gradually increased with storage time (Figure 1B). The TBARS values of cooked beef loin and chicken thigh meat were approximately 2 times greater than those of pork loin and chicken breast meat after 7 d of storage. Our TBARS results of cooked meats were different from those of Rhee and others (1996), who reported that the TBARS of cooked chicken thigh meat was 2-fold higher than those of beef, pork, and chicken breast meat.

#### Total fat content and fatty acid composition

Beef loin and chicken thigh meat had higher total fat content than pork loin and chicken breast meat in both raw and cooked meats (Table 1). The composition of fat is more important than the amount of fat in meat because the susceptibility of muscle lipid-to-lipid oxidation depends upon the degree of polyunsaturation in fatty acids. Wagner and others (1994) demonstrated that the number of bis-allylic positions in lipid molecules determines their susceptibility to lipid oxidation, and the rate of lipid oxidation increased exponentially with the number of bis-allylic positions, although lipid chain length had no relationship with the rate of radical formation. The amount of total PUFAs in raw chicken thigh meat (2.44 g/100 g meat) was much higher than those of beef loin, pork loin, and chicken breast meat (0.74, 0.72, and 0.69 g/100 g meat, respectively), according to Table 1. It is assumed that most PUFAs in total fat are stored in adipose tissue as a TG form in meat. TG is the dominant lipid class (Ahn and others 1993a), and most TG is stored in adipose tissues where the amounts and accessibility of prooxidants such as myoglobin are very low. The PUFA contents (percent) in TG of chicken thigh meat also were significantly higher than those of pork and beef loin (Table 2). However, the TBARS values of raw chicken thigh meat did not change during storage

Table 1—Total fat content (percent of meat) and fatty acid composition of raw and cooked chicken breast, chicken thigh, pork loin, and beef loin.

	Chicken breast	Chicken thigh	Pork Ioin	Beef loin	SEM
Raw meat					
Total fat (%)	1.77 <sup>b</sup>	6.32ª	3.02 <sup>b</sup>	7.93ª	0.42
		Percent of	of total fat		
SFA	31.62 <sup>cz</sup>	27.92 <sup>cz</sup>	36.84 <sup>by</sup>	43.71 <sup>ay</sup>	1.06
MUFA	29.34cz	33.44cy	39.46 <sup>by</sup>	46.91ay	1.10
PUFA	39.03ay	38.64 <sup>ax</sup>	23.69bz	9.39cz	0.85
Total UFA	68.38 <sup>ax</sup>	72.08aw	63.16 <sup>bx</sup>	56.29 <sup>cx</sup>	1.06
SEM	1.15	0.40	1.08	1.24	
Cooked meat					
Total fat (%)	1.97 <sup>d</sup>	6.97 <sup>b</sup>	3.76°	8.53ª	0.32
		Percent of	of total fat		
SFA	32.22bcz	29.17 <sup>cz</sup>	36.21 <sup>by</sup>	42.42ay	1.13
MUFA	28.20 <sup>cy</sup>	30.84 <sup>bcz</sup>	35.55 <sup>by</sup>	43.88ay	1.41
PUFA	39.58 <sup>ayx</sup>	39.99 <sup>ay</sup>	28.24 <sup>bz</sup>	13.70 <sup>cz</sup>	1.99
Total UFA	67.78 <sup>abw</sup>	70.83 <sup>ax</sup>	63.79 <sup>bx</sup>	57.58 <sup>cx</sup>	1.13
SEM	0.66	1.01	1.19	2.38	

and Means with different letters within the same row are significantly different (P < 0.05). n = 4.

(Figure 1A), suggesting that PUFA content in total fat may not be proportionately related to the lipid oxidation in raw meats.

Igene and Pearson (1979) and Pikul and others (1984) suggested that PLs are primarily responsible for rancidity and warmed-over flavor (WOF) development in raw and cooked meat, because the high susceptibility of PL to oxidative changes is attributed not only to its high PUFA content but also to its omnipresence in cell membranes where prooxidants in the cell matrix can have easy access. Pikul and others (1984) suggested that the PL fraction contributed about 90% of the malonaldehyde measured in total fat from chicken meat. Fatty acid compositions of PC and PE of raw

W=2 Means with different letters within the same column are significantly different (P < 0.05).

SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; UFA = unsaturated fatty acid (MUFA + PUFA); SEM = standard error of the means.

and cooked meat showed that PEs of all raw and cooked meats were more polyunsaturated than PCs (Table 3 and 4). Yin and Faustman (1993) reported that PE was more susceptible to lipid oxidation than PC. Also, PE was suggested to be the major contributor to the development of warmed-over flavor in cooked meat (Igene and Pearson 1979; Igene and others 1981). PUFA content in PE of raw beef loin was similar to that of pork loin and chicken thigh meats (Table 3), although that of raw beef loin was slightly lower than those of pork loin and chicken thigh meats (Table 4). This result suggested that PUFA content in PE and PC was not the main factor affecting different TBARS changes between raw beef loin and other meats (Figure 1). Therefore, oxidative factors other than fatty acid composition should be involved in high TBARS values of raw beef loin compared with other meats.

On the other hand, PUFA content in cooked meat could be an important factor affecting the rate of lipid oxidation because heat disrupts the cell membrane structure to increase the accessibility of prooxidants to PUFA in TG (Min and Ahn 2005), although prooxidants still have easier access to PUFAs in cell membrane

Table 2-Fatty acid composition of triglycerides (TGs) from raw and cooked chicken breast, chicken thigh, pork loin, and beef loin.

•					
	Chicken breast	Chicken thigh	Pork Ioin	Beef loin	SEM
	F	Percent of tot	al triglyceric	les	_
Raw meat					
SFA	30.21 <sup>cz</sup>	27.07 <sup>cz</sup>	44.35 <sup>by</sup>	51.58 <sup>ax</sup>	1.35
MUFA	41.21 <sup>by</sup>	40.80 <sup>bx</sup>	49.93 <sup>ax</sup>	46.92ax	1.32
PUFA	28.58 <sup>bz</sup>	32.12ay	5.72 <sup>cz</sup>	1.50 <sup>dy</sup>	0.76
Total UFA	69.79 <sup>ax</sup>	72.93 <sup>aw</sup>	55.65 <sup>bw</sup>	48.42 <sup>cx</sup>	1.35
SEM	0.56	0.71	1.00	2.04	
Cooked meat					
SFA	31.27cz	28.20cz	44.60 <sup>by</sup>	52.06ax	1.00
MUFA	39.89 <sup>by</sup>	41.39bx	50.52ax	46.49ax	1.00
PUFA	28.84az	30.41ay	4.88 <sup>bz</sup>	1.44 <sup>cy</sup>	0.53
Total UFA	68.73 <sup>ax</sup>	71.80 <sup>aw</sup>	55.40 <sup>bw</sup>	47.94 <sup>cx</sup>	1.00
SEM	0.60	0.41	0.94	1.36	

a-d Means with different letters within the same row are significantly different

Table 3 - Fatty acid composition of phosphatidylcholine (PC) from raw and cooked chicken breast, chicken thigh, pork loin, and beef loin.

	Chicken	Chicken	Pork	Beef	OEM			
	breast	thigh	loin	loin	SEM			
	Pe	Percent of phosphatidylcholine						
Raw meat		•	. ,					
SFA	48.34 <sup>ax</sup>	48.77 <sup>ax</sup>	44.88 <sup>abx</sup>	41.23 <sup>by</sup>	0.99			
MUFA	21.73 <sup>bz</sup>	18.78 <sup>bz</sup>	20.78 <sup>bz</sup>	31.17 <sup>az</sup>	0.96			
PUFA	29.93 <sup>bcy</sup>	32.45 <sup>aby</sup>	34.34 <sup>ay</sup>	27.60 <sup>cz</sup>	0.94			
Total UFA	51.66 <sup>bw</sup>	51.23 <sup>bx</sup>	55.12abw	58.77 <sup>ax</sup>	0.99			
SEM	0.54	1.07	0.82	1.30				
Cooked meat								
SFA	49.21ax	46.72abx	43.49bcx	37.57 <sup>cx</sup>	0.80			
MUFA	22.36 <sup>bz</sup>	19.05 <sup>cz</sup>	21.20 <sup>bcz</sup>	33.90 <sup>ay</sup>	0.77			
PUFA	28.43 <sup>by</sup>	34.23 <sup>ay</sup>	35.31 <sup>ay</sup>	28.53 <sup>bz</sup>	0.45			
Total UFA	50.79 <sup>cx</sup>	53.28 <sup>bcw</sup>	56.51 <sup>abw</sup>	62.43 <sup>aw</sup>	0.80			
SEM	0.46	0.23	0.31	0.49				

<sup>&</sup>lt;sup>a-d</sup>Means with different letters within the same row are significantly different

than PUFAs of TG in adipose tissues. The amount of total PUFAs in cooked chicken thigh meat (2.79 g/100 g meat; Table 1) was the highest among the cooked meats and might be highly related to the rapid increase in its TBARS value during storage (Figure 1B). Rhee and others (1996) also reported that the high amount of PUFAs was responsible for the high lipid oxidation rates in cooked chicken thigh meat.

# Total, heme, and nonheme iron

As expected, total iron content for raw meats was the highest in beef loin, followed by chicken thigh meat, chicken breast meat, and pork loin (Table 5). Heme iron content in raw beef loin was also the highest among the meats. The major source of heme iron in beef is myoglobin, which accounts for over 90% of heme proteins present in beef (Hazell 1982). Myoglobin seems to be a core compound in the lipid oxidation process of meat because myoglobin in the presence of H<sub>2</sub>O<sub>2</sub> or LOOH can be converted to ferrylmyoglobin and serve as a major source of hematin and free ionic iron, all of which able to initiate and catalyze propagation of lipid oxidation (Min and Ahn 2005).

The content of nonheme iron in raw chicken thigh meat was higher than that of other meats at day 0; no changes in nonheme iron content in raw pork loin and chicken breast and thigh meats during storage were observed (Table 6). However, nonheme iron content in raw beef loin significantly increased with storage time, and the amount at day 7 was approximately 2 times as high as that

Table 4 **–** Fatty acid composition of phosphatidylethanolamine (PE) from raw and cooked chicken breast, chicken thigh, pork loin, and beef loin.

	Chicken breast	Chicken thigh	Pork Ioin	Beef loin	SEM				
	Perce	Percent of phosphatidylethanolamine							
Raw meat			,						
SFA	37.16 <sup>ay</sup>	35.54 <sup>aby</sup>	30.66 <sup>by</sup>	36.10 <sup>aby</sup>	1.51				
MUFA	22.96az	16.49 <sup>bz</sup>	15.84 <sup>bz</sup>	16.73 <sup>bz</sup>	0.96				
PUFA	39.88 <sup>by</sup>	47.97 <sup>abx</sup>	53.50 <sup>ax</sup>	47.16 <sup>abx</sup>	2.04				
Total UFA	62.84 <sup>bx</sup>	64.46 <sup>abw</sup>	69.34 <sup>aw</sup>	63.90 <sup>abw</sup>	1.51				
SEM	1.93	0.30	1.51	1.89					
Cooked meat									
SFA	35.85 <sup>ay</sup>	34.30 <sup>ay</sup>	27.81 <sup>cy</sup>	31.07 <sup>by</sup>	0.65				
MUFA	22.13 <sup>az</sup>	15.96 <sup>cz</sup>	14.59 <sup>cz</sup>	18.80 <sup>bz</sup>	0.44				
PUFA	42.02 <sup>cx</sup>	49.74 <sup>bx</sup>	57.60 <sup>ax</sup>	50.13 <sup>bx</sup>	0.77				
Total UFA	64.15 <sup>cw</sup>	65.70 <sup>cw</sup>	72.19 <sup>aw</sup>	68.93 <sup>bw</sup>	0.65				
SEM	0.41	0.17	0.94	0.74					

a-d Means with different letters within the same row are significantly different

Table 5 - Iron content of raw and cooked chicken breast, chicken thigh, pork loin, and beef loin at day 0.

	Chicken breast	Chicken thigh	Pork loin	Beef loin	SEM
	Microgra	am iron per	gram mea	at	
Raw meat	· ·	•	ŭ		
Total iron	6.17°	9.45 <sup>b</sup>	5.53°	19.51ª	1.46
Heme iron	4.87 <sup>b</sup>	7.54 <sup>b</sup>	4.60 <sup>b</sup>	18.39ª	1.41
Nonheme iron	1.30 <sup>b</sup>	1.91ª	0.93°	1.13 <sup>bc</sup>	0.15
Cooked meat					
Total iron	6.08°	9.70 <sup>b</sup>	6.02°	24.14 <sup>a</sup>	0.94
Heme iron	4.33°	6.83 <sup>b</sup>	4.68°	21.73ª	0.89
Nonheme iron	1.75 <sup>b</sup>	2.88ª	1.33 <sup>b</sup>	2.41ª	0.23

a-c Means with different letters within the same row are significantly different (P < 0.05)

<sup>(</sup>P< 0.05). n= 4. W=2Means with different letters within the same column are significantly different

SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; UFA = unsaturated fatty acid; SEM = standard error

<sup>(</sup>P < 0.05). n = 4. W=2 Means with different letters within the same column are significantly different

SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; UFA = unsaturated fatty acid; SEM = standard error of the means.

<sup>(</sup>P < 0.05). n = 4.

W<sup>2</sup> Means with different letters within the same column are significantly different letters within the same column are significantly different letters. (P < 0.05). SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; UFA = unsaturated fatty acid; SEM = standard error of the means.

SEM = standard error of the means. n = 4.

at day 0. The increase of nonheme iron in raw beef loin during storage could be caused by the release of iron from heme pigments, especially myoglobin in the presence of H2O2 or lipid hydroperoxide (Min and Ahn 2005). However, our study (Min 2006) indicated that most of nonheme iron released before 5 d of storage in the refrigerator was accumulated in the water-insoluble fraction of beef loin. It was assumed that hemosiderin in the water-insoluble fraction chelated the free ionic iron released from myoglobin and, thus, inactivated its catalytic activity for the lipid oxidation process. Hemosiderin is a water-insoluble complex of iron, other metals, and proteins and is considered a product of ferritin decomposition or polymerization (Decker and Hultin 1992). Prasad and others (1989) showed that hematin was released from myoglobin before the release of free ionic iron in the presence of H<sub>2</sub>O<sub>2</sub> and the amount of hematin released from metmyoglobin during incubation was greater than that of released free ionic iron. Hematin can react with  $H_2O_2$  or LOOH to form ferrylhematin (Fe<sup>4+</sup> = O), which can initiate and propagate lipid oxidation (Dix and Marnett 1985; Kim and Sevanian 1991). Therefore, ferrylmyoglobin and/or hematin, rather than free ionic ion, could be primarily responsible for higher TBARS values in raw beef loin than in other meats during storage (Figure 1A).

Heating increased nonheme iron content in meats from all animal species as previously reported (Apte and Morrissey 1987; Rhee and others 1996). Cooked beef loin and chicken thigh meat had higher nonheme iron content than cooked pork loin and chicken breast meat, but nonheme iron content was not changed during storage in cooked pork loin, chicken breast meat, and chicken thigh meat. Myoglobin that should have been the source of nonheme iron increased in cooked beef loin. However, the catalytic activity of free ionic iron depends on the presence of reducing capacity in meat from ferric to ferrous ion because ferric iron cannot catalyze lipid oxidation (Halliwell and Gutteridge 1999). Ahn and Kim (1998) indicated that the status of free ionic iron is more important than the amount of ionic iron for the development of lipid oxidation in meat. Therefore, the increased free ionic iron content by heating and storage, as well as the level of ferric ion reducing capacity, played important roles for the increased TBARS values in cooked beef loin (Figure 1A and 1B).

#### Ferric ion reducing capacity (FRC)

The ability of antioxidant compounds to reduce ferric to ferrous ion, namely, the ferric-reducing antioxidant power (FRAP)

Table 6-Nonheme iron content of raw and cooked chicken breast, chicken thigh, pork loin, and beef loin during storage at 4  $^{\circ}$ C.

Storage	Chicken breast	Chicken thigh	Pork Ioin	Beef loin	SEM
	Microg	ram nonhem	e iron per g	ram meat	
Raw meat	_				
0 d	1.30 <sup>b</sup>	1.91ª	$0.93^{\circ}$	1.13 <sup>bcy</sup>	0.15
3 d	1.20 <sup>b</sup>	1.74 <sup>a</sup>	1.12 <sup>b</sup>	1.35 <sup>aby</sup>	0.09
7 d	1.08 <sup>b</sup>	1.74 <sup>b</sup>	1.20 <sup>b</sup>	2.13 <sup>ax</sup>	0.10
SEM	0.10	0.10	0.07	0.10	
Cooked mea	at				
0 d	1.75 <sup>b</sup>	2.88a	1.33 <sup>b</sup>	2.41ay	0.23
3 d	1.35°	2.66 <sup>b</sup>	1.89°	3.70 <sup>ax</sup>	0.18
7 d	1.50°	2.77 <sup>b</sup>	1.89°	4.82ax	0.36
SEM	0.11	0.10	0.10	0.31	

a-c Means with different letters within the same row are significantly different

assay, had been used to evaluate the antioxidant activity in biological systems (Benzie and Strain 1999). Various reducing compounds, including ascorbic acid, NAD(P)H, and thiol compounds such as glutathione (GSH), are present in biological cells and may be primarily responsible for ferric ion reducing capacity (FRC) of meat. Ascorbic acid is an important biological reducing agent and can serve as an electron donor in free radical-mediated oxidative processes (Buettner and Jurkiewicz 1996). The amount of reducing compounds in turkey muscles is about 3 mg ascorbic acid equivalent/100 g fresh meat, and 80% of the reducing compounds were ascorbic acid (Kanner and others 1991). In general, ascorbic acid can serve as both an antioxidant and a prooxidant, depending on the concentration of ascorbic acid present. It has been suggested that ascorbic acid at low concentration is most likely to promote lipid oxidation in muscle tissues by reducing Fe(III) to Fe(II), which can catalyze the formation of hydroxyl radical (•OH) in the ascorbic acid-assisted Fenton reaction (Decker and Hultin 1992), whereas at high concentration, it tends to inhibit lipid oxidation by regenerating antioxidants such as  $\alpha$ -tocopherol in cell membrane, directly reducing some of the lipid peroxyl radicals to hydroperoxides (Halliwell and Gutteridge 1999) and reducing ferrylmyoglobin (Giulivi and Cadenas 1993; Kroger-Ohlsen and Skibsted 1997). The concentration effects of ascorbic acid on lipid oxidation also vary, depending upon the concentration of iron present (Decker and Hultin 1992). Therefore, we assumed that the FRC observed in this study could be considered either an antioxidant or prooxidant activity in meat, depending on the concentration of free ionic

In raw meat, FRCs of chicken breast and thigh were significantly higher than those of pork and beef loin (Table 7). The FRCs of raw chicken breast and thigh meats decreased with storage, whereas those of raw beef and pork loin did not change. This suggested that significant amounts of FRCs of raw chicken breast and thigh were storage-unstable, but most of FRCs in raw beef and pork loin were storage-stable. Also, it is assumed that high FRC and low concentration of nonheme iron are responsible for low TBARS values in raw chicken breast and thigh during storage.

Heating reduced the FRCs of chicken breast and thigh more than those of pork and beef loins. Generally, ascorbic acid is readily decomposed by heating and, thus, most of FRCs present in cooked meat may not be ascorbic acid. Also, the changes in the amounts of FRCs of pork and beef loin before and after cooking were small, indicating that raw pork and beef loin contained only small amounts of unstable reducing agents such as ascorbic acid.

Table 7 – Ferric ion reducing capacity of raw and cooked chicken breast, chicken thigh, pork loin, and beef loin during storage at 4  $^{\circ}$ C.

Storage	Chicken breast	Chicken thigh	Pork loin	Beef loin	SEM		
	Microgram ascorbic acid equivalent per gram meat						
Raw meat	J			Ü			
0 d	22.23ax	21.50 <sup>ax</sup>	6.75 <sup>b</sup>	7.03 <sup>b</sup>	0.56		
3 d	13.84 <sup>ay</sup>	11.71 <sup>ay</sup>	7.45 <sup>b</sup>	7.72 <sup>b</sup>	0.66		
7 d	11.27 <sup>ay</sup>	10.71 <sup>ay</sup>	6.24 <sup>b</sup>	4.43°	0.37		
SEM	0.74	0.60	0.43	0.28			
Cooked me	eat						
0 d	3.22 <sup>dx</sup>	4.47 <sup>bx</sup>	3.96 <sup>cx</sup>	6.18 <sup>a</sup>	0.10		
3 d	2.71 <sup>cy</sup>	2.89 <sup>cy</sup>	3.44 <sup>bxy</sup>	5.77 <sup>a</sup>	0.08		
7 d	2.28 <sup>bz</sup>	2.90 <sup>by</sup>	3.19 <sup>by</sup>	5.56ª	0.31		
SEM	0.08	0.12	0.17	0.32			

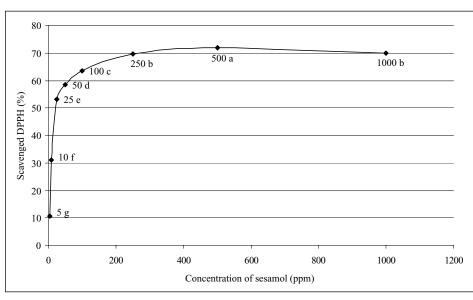
 $<sup>^{</sup>a-c}$ Means with different letters within the same row are significantly different (P < 0.05)

<sup>(</sup>P < 0.05)

 $<sup>\</sup>frac{\lambda}{2}$  Means with different letters within the same column are significantly different (P < 0.05).

SEM = standard error of the means. n = 4.

<sup>(</sup>P < 0.05).  $^{\times -2}$ Means with different letters within the same column are significantly different (P < 0.05). SEM = standard error of the means. n = 4.



1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of sesamol as a reference. The numbers next to the diamonds in this figure represent the concentrations of sesamol used in the experiment. Means with different letters (a to g) within concentrations of sesamol are significantly different (P < 0.05). n = 4.

Heat-stable FRC was detected in all cooked meats. Ferric ion can hardly catalyze lipid oxidation without reducing compounds (Ahn and Kim 1998). Reducing agents can act as prooxidants when their amounts are relatively low. Cooking decreased the FRC but increased nonheme iron in meat, resulting in comparatively lower FRC in cooked meat than raw meat. Therefore, heat-stable FRC may be primarily responsible for the regeneration of Fe(II) to increase TBARS in cooked meat during storage. The heat-stable FRC of beef loin was the highest and very stable during storage, indicating that high "heat-stable" FRC and nonheme iron were responsible for the rapid TBARS increase in cooked beef loin during storage. Although heat-stable FRC in cooked chicken thigh was lower than that in cooked beef loin, their TBARS values after 7 d of storage were similar. This was attributed to high amounts of PUFAs in cooked chicken compared with beef loin. Heating disrupts membrane barriers in meat and facilitates the access of prooxidants to PUFAs in adipose tissues (Min and Ahn 2005).

#### Free radical scavenging activities

As the concentration of sesamol, the reference for free radical scavenging activities, increased to 500 ppm, the scavenged DPPH increased exponentially to approximately 72% of the initial amount (Figure 2). The scavenged DPPH increased dramatically to about 53% in the presence of low concentrations of sesamol (up to 25 ppm), but the rate of scavenged DPPH decreased gradually over 25 ppm up to 500 ppm of sesamol. The scavenging of DPPH also occurs at sesamol concentrations above 500 ppm but at slower rates. This phenomenon could be caused by the absence of a reducing compound, a regenerator of sesamol from sesamol radical. As the concentration of sesamol increases, more sesamol radicals can be generated and accumulated by scavenging 1 electron from DPPH radical, unless they are regenerated to sesamol by reducing compounds. Consequently, the accumulated sesamol radicals could compete with residual DPPH radical to react with remaining sesamol, resulting in the reduction of DPPH radical scavenging rate. Therefore, the presence of reducing compounds may be necessary for maximizing the free radical scavenging ability of antioxidants. It is suggested that  $\alpha$ -tocopherol radical can react with PUFAs without reducing compounds, although the rate constant of  $\alpha$ -tocopherol radical with PUFA is much smaller than that of reactive oxygen species (ROS; Halliwell and Gutteridge 1999).

Table 8 – 1,1-Diphenyl-2-picrylhydrazyl (DPPH) scavenging activity of raw and cooked chicken breast, chicken thigh, pork loin, and beef loin during storage at

Storage	Chicken breast	Chicken thigh	Pork loin	Beef loin	SEM
		Scavenged	DPPH (%)		
Raw meat		•	, ,		
0 d	88.43 <sup>ax</sup>	86.18 <sup>ax</sup>	69.24 <sup>b</sup>	61.92 <sup>b</sup>	4.43
3 d	79.02 <sup>ay</sup>	71.38 <sup>aby</sup>	63.77 <sup>b</sup>	63.97 <sup>b</sup>	4.59
7 d	53.21 <sup>abz</sup>	43.51 <sup>bz</sup>	60.11a	58.20a	4.73
SEM	3.01	2.70	5.07	6.48	
Cooked me	eat				
0 d	83.15 <sup>ax</sup>	84.43 <sup>ax</sup>	59.87 <sup>bx</sup>	67.44 <sup>bx</sup>	5.15
3 d	62.73 <sup>ay</sup>	65.58 <sup>ay</sup>	45.89 <sup>by</sup>	56.98ay	4.13
7 d	54.86 <sup>az</sup>	56.44 <sup>az</sup>	42.15 <sup>by</sup>	44.88 <sup>bz</sup>	4.18
SEM	3.90	2.10	6.10	4.95	

a-c Means with different letters within the same row are significantly different

(P < 0.05). X=2 Means with different letters within the same column are significantly different (P < 0.05). SEM = standard error of the means. n = 4.

In raw meat, the DPPH radical scavenging activities of chicken breast (88.43% and 79.02%) and thigh (86.18% and 71.38%) meat at days 0 and 3, respectively, were significantly higher than those of pork and beef loin, and were similar to or greater than those of 500 ppm sesamol (71.96%) (Table 8 and Figure 2). The higher amounts of reducing compounds in raw chicken breast and thigh meats compared with those in raw pork and beef loins (Table 7) could be partially responsible for their higher DPPH radical scavenging activities. In addition, chicken meats are reported to contain more histidine-containing dipeptides such as carnosine and anserine, which have antioxidant activities, than beef and pork (Chan and Decker 1994). DPPH radical scavenging activities in raw beef and pork loin did not change during storage as FRC in them (Table 7). We assumed that the DPPH radical scavenging activities in raw beef and pork loin were storage-stable and were closely related to storage-stable FRC. In addition, DPPH radical scavenging activity of raw beef loin during storage was similar to that of raw pork loin, although TBARS values increased only in raw beef loin during storage. It seemed that the amounts of antioxidants in raw pork loin were large enough to prevent lipid oxidation. The levels of prooxidant factors such as heme and nonheme iron in pork loin (Table 6) were lower than those in beef loin. In addition, pork has significantly higher catalase activities than beef and

chicken (Rhee and others 1996), which can decrease the production of ferrylmyoglobin.

DPPH radical scavenging activities of all meats were not affected by heating and decreased significantly during storage. Compounds that could scavenge DPPH radicals in all meats seemed to be heat-stable. Higher amounts of carnosine and anserine in chicken breast and thigh meats (Chan and Decker 1994) could be responsible for the higher DPPH radical scavenging activities in cooked chicken and thigh meats. Despite the presence of DPPH radical scavenging activities in cooked chicken thigh meat and beef loin, TBARS values increased dramatically in those meats during storage (Figure 1B). It is assumed that the power of prooxidant factors such as levels of nonheme and heme iron and/or PUFAs exceeded the antioxidant capacities in cooked chicken thigh meat and beef loin.

# Lipoxygenase-like activities

Lipoxygenase activity is essential for the biosynthesis of eicosanoids from arachidonic acid in cell membrane (Garrett and Grisham 1999). Lipoxygenases have been identified in various mammalian tissues, including skeletal muscles (Grossman and others 1988; Yamamoto 1992; Gata and others 1996). Lipoxygenase is capable of direct oxygenation of PUFAs even in PL bound to membrane to generate lipid hydroperoxides. Therefore, lipoxygenase can be involved in the initiation of lipid oxidation in meat. However, lipoxygenase activities measured in this study (Table 9) were greater than those of other reports (Grossman and others 1988; Gata and others 1996), and part of the activities could be lipoxygenase-like activities rather than true lipoxygenase activities. Ferrylmyoglobin generated by the interaction of metmyoglobin and H2O2 can directly abstract a hydrogen atom from a PUFA and generate lipid hydroperoxides in a similar manner to lipoxygenase (Rao and others 1994).

Raw beef loin and chicken thigh meat showed higher lipoxygenase-like activities than chicken breast and pork loin (Table 9). These trends are highly related to total iron contents, especially heme iron content in meat (Table 5). Lower heme iron contents in chicken breast meat and pork loin may be responsible for their lower lipoxygenase-like activities. The initial lipoxygenase-like activities in raw chicken thigh meat were similar to those in raw beef loin, but decreased during storage. The lipoxygenase-like activities in beef loin increased considerably during storage. The differences in the changes of lipoxygenase-like activities

Table 9-Lipoxygenase-like activity of raw and cooked chicken breast, chicken thigh, pork loin, and beef loin during storage at 4  $^{\circ}$ C.

Storage	Chicken breast	Chicken thigh	Pork Ioin	Beef loin	SEM				
		Unit per gram meat							
Raw meat									
0 d	1.64 <sup>bx</sup>	4.56 <sup>ax</sup>	0.63 <sup>b</sup>	5.66az	0.31				
3 d	1.47 <sup>cx</sup>	3.89 <sup>by</sup>	$0.62^{d}$	8.69 <sup>ay</sup>	0.17				
7 d	0.76 <sup>cy</sup>	2.27 <sup>bz</sup>	0.59°	12.89 <sup>ax</sup>	0.17				
SEM	0.09	0.12	0.21	0.42					
Cooked me	eat								
0 d	0.21bx	0.09°	0.14°	0.34ª	0.01				
3 d	0.23ax	0.14 <sup>b</sup>	0.14 <sup>b</sup>	0.31ª	0.02				
7 d	0.09 <sup>cy</sup>	0.14°	0.25 <sup>b</sup>	0.39ª	0.02				
SEM	0.02	0.01	0.01	0.02					

 $<sup>^{\</sup>rm a-c}$  Means with different letters within the same row are significantly different (P<0.05).  $^{\rm x-z}$  Means with different letters within the same column are significantly different

Units per gram meat is defined as the amount of enzyme per gram meat catalyzing the formation of 1  $\mu$ mol of hydroperoxide per minute.

during storage between chicken thigh meat and beef loin could be attributed to the difference in the concentration of reducing compounds, especially ascorbic acid (Table 7). Because ascorbic acid can reduce ferrylmyoglobin (Giulivi and Cadenas 1993; Kroger-Ohlsen and Skibsted 1997), relatively high lipoxygenase-like activity in raw beef loin should be attributed to high concentration of myoglobin and low concentration of reducing compounds. In addition, the lipoxygenase-like activities in meat decreased dramatically after cooking, indicating that these activities were heat-labile and were not from •OH generated from the Fenton reaction. Therefore, ferrylmyoglobin could be primarily responsible for the lipoxygenase-like activities measured in this study.

# **Conclusions**

Raw beef loin was the most susceptible to lipid oxidation among meats from different animal species during storage. The contents of myoglobin, ferric ion reducing capacity (FRC), and DPPH radical scavenging activity were the primary determinants for the differences in susceptibility of raw meats to lipid oxidation. Cooked pork loin and chicken breast meat were less susceptible to lipid oxidation than cooked beef loin and chicken thigh meat, although the TBARS values increased during storage in all meat species. The contents of free ionic iron and heat-stable FRC played a key role on the development of lipid oxidation in cooked meat; and the amount of PUFAs in cooked meat was also related to lipid oxidation in cooked meat when sufficient amount of free ionic iron was present.

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 $<sup>^{\</sup>times -2}$ Means with different letters within the same column are significantly different (P < 0.05). SEM = standard error of the means. n = 4. Units per gram meat is defined as the amount of enzyme per gram meat

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